510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K030702

B. Analyte:
IgM Anti-β_2 Glycoprotein I

C. Type of Test:
Semi-quantitative (ELISA)

D. Applicant:
IMMCO Diagnostics

E. Proprietary and Established Names:
ImmuLisa Anti-β_2 GPI IgM ELISA

F. Regulatory Information:

1. Regulation section:
   21 CFR 866.5660 Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MSV System, Test, Antibodies, B2-Glycoprotein I (B2-GPI)
4. Panel:
   82 Immunology

G. Intended Use:

1. Indication(s) for use:
   An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgM antibodies to β_2 - GPI, as an aid in assessing the risk of thrombosis in patients with Systemic Lupus Erythomatosus (SLE) or lupus like disorders.

2. Special condition for use statement(s):
   NA
3. Special instrument Requirements:
   Microplate reader capable of reading absorbance values at 405 nm. If dual microplate reader is available, the reference filter should be set at 600-650 nm

H. Device Description:
The ImmuLisa Anti-Beta$_2$ Glycoprotein I (β_2GPI) IgM ELISA test kit contains a microplate coated with β_2GPI, calibrators, positive and negative controls, enzyme-labeled conjugate, substrate and substrate stop solution, buffered diluent and wash buffer.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA Quanta Lite anti-β_2 GPI IgM Antibody Test kit

2. Predicate K number(s):
   K973014
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Detection and semi-quantitation of IgM antibodies to B2-GPI	Same
Methodology	Enzyme linked immunosorbent assay	Same
Sample Dilution	1:101	Same
Differences		
Item	**Device**	**Predicate**
Conjugate	Alkaline Phosphatase labeled	Horseradish peroxidase labeled
Calibrators	Set of 4 pre-diluted calibrators	Set of 5 pre-diluted calibrators
Substrate	p-NPP	TMB
Absorbance	405 nm	450 nm

J. Standard/Guidance Document Referenced (if applicable):

NA

K. Test Principle:

The test is performed as a solid phase immunoassay (ELISA) in β_2 - GPI coated microwells. Controls, calibrators and patient serum samples are incubated in the antigen coated microwells to allow antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding an enzyme labeled anti-human IgM conjugate to the microwells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme-labeled conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
      Three samples with known concentrations of anti β_2-GPI were assayed in 10 replicates over a two week period. This study included negatives (less than 20 EU/mL), weak positives and other values extended through the high-end calibrator range of 160 EU/mL. Intra- and inter-assay coefficients of variation (CV) were as follows:

	Sample 1	Sample 2	Sample 3
Inter-assay CV	8.2%	13.0%	16.8%
Intra-assay CV	10.3%	4.9%	8.3%

b. Linearity/assay reportable range:
Linearity was established in a variety of calibrator studies during development. Absorbance values versus calibration were established in a linear to linear plot using known values of calibrators using standard statistical methods. The r-squared value for the curve is 0.996.
Graph showed linearity up to 236 EU/mL

c. Traceability (controls, calibrators, or method):
Positive controls and calibrators were derived from serum obtained from various commercial plasma centers. Samples were selected on the basis of the specific antibody reactivity and the concentration. For assignment of values, the samples were tested at various dilutions on at least two different lots of the β_2-GPI antigen coated plates

d. Detection limit:
-1.319 EU/mL
The limits of detection fall well below the established cutoff.

e. Analytical specificity:
Interference studies were provided using positive specimens diluted into hemolyzed and lipemic serum. Slight depression of O.D. was noted in specimens diluted in normal serum. The product insert included a statement warning users against the use of grossly hemolyzed or lipemic sera.

f. Assay cut-off:
Less than 20 EU/mL. Cut-off data is provided. It was calculated using Mean +2 standard deviations.

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:
The IMMCO ImmuLisa Anti-Beta$_2$ Glycoprotein I (β_2GPI) IgM assay was compared with INOVA Anti β_2-GPI IgM ELISA (K973014) as a predicate device. A range of positives with antibody levels spanning from low positive to high positive values, disease controls from patients with syphilis, and normal patients were tested.

	ImmuLisa Anti B2GPI IgM		
	Positive	Negative	Total
INOVA Anti-B2-GPI IgM ELISA	16	2	18
	1	49	50
	17	51	68

Relative Agreement: 96%
Relative Sensitivity: 89%
Relative Specificity: 98%

b. Matrix comparison:
Both tests are for human serum only

3. Clinical studies:
   a. Clinical sensitivity:
   NA
   b. Clinical specificity:
   It is known that patients suffering from infectious diseases like syphilis have cardiolipin antibodies and lupus anti-coagulant. To test the clinical specificity of the assay, sera from patients with syphilis were tested for the presence of anti-cardiolipin and anti-B2GPI antibodies. The study suggests that B2-GPI antibodies are more specific than anti-cardiolipin antibodies as only a small percentage of the patients with syphilis are positive for B2-GPI.

   c. Other clinical supportive data (when a and b are not applicable):
   Clinical studies of anti-B2GPI antibodies have been predicated upon the relationship between the occurrence of B2GPI antibodies and anti-cardiolipin antibodies in Anti-phospholipid Syndrome (APS) patient populations. A reference demonstrating the correlation is provided.
4. Clinical cut-off:
   Negative <20
   Borderline 20-25
   Positive >25
5. Expected values/Reference range:
   The normal range was established by testing 64 serum samples from apparently healthy donors obtained from the local Red Cross.
   The mean plus three SD of the mean of this normal was used to determine the cut off of the normal to the abnormal. This was assigned an arbitrary unit value of 20 EU/mL

M. Conclusion:

Based on the review of the information provided in this 510(k), the ImmuLisa Anti-Beta$_2$ Glycoprotein I (β_2GPI) IgM appears to be **Substantially Equivalent** to devices

regulated under 21CFR 866.5660, Multiple Autoantibodies Immunological Test System, Product code MSV, Class II.